REMARKS:

In this Amendment, the currently pending claims are claims 196-259. Claims 1-195 have been cancelled without prejudice or disclaimer and are replaced by new claims 196-259 herein. The specification has been amended for clarity as indicated.

The new claims are supported by the application and previously-filed claims, and no new matter has been introduced into the application by virtue of the new claims. Specifically, support for new claim 196 is found in the instant specification, *inter alia*, at page 17, lines 1-11; at page 30, lines 13-25; at page 42, lines 10-28;at page 98 *et seq.*, Example 11; at page 99, lines 3-4 and at page 102, lines 2-4. Support for new claims 202, 218, 234 and 250 is found in the instant specification, *inter alia*, on page 24, line 7. Support for new claims 203, 219, 235 and 251 is found in the instant specification, *inter alia*, on page 82, lines 13-25 to p. 83, line 1 and Table 4. Support for new claims 204, 220, 236 and 252 is found in the instant specification, *inter alia,* on p. 84, lines 25-28 to p. 85, lines 1-9; and in Table 5, p. 85 of the instant specification. The specification also teaches that iNOS and IRAP are expressed in MDCs following introduction of a vector harboring a polynucleotide sequence encoding iNOS and IRAP into the cells. (Example 7, pp. 73-77; p. 70, lines 14-28 of the instant specification).

The Examiner's attention is again drawn to the change in docket number for this application. Specifically, the docket number is changed from "2710-4007US1" to "PIT-010". It is respectfully requested that the new docket number be docketed for this application in the U.S. Patent and Trademark Office.

I. The Enablement Rejection

The final Office Action dated July 9, 2003 has been considered as if it pertained to newly presented claims 196-259. Thus, this Amendment is responsive to the final Office Action as if it applied to the new claims.

Claims 119-195 stand rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art ... to make and/or use the invention.

Applicants respectfully disagree with this rejection and submit that the claims as presented herein satisfy the requirements of §112, first paragraph, and satisfy the Examiner's concerns as set forth on pages 2-7 of the 07/09/2003 office action.

The Examiner remarks on page 5 that "... considering the instant specification, the applicant fails to disclose that bulking of the sphincter muscle alone would lead to the treatment of urinary stress incontinence, since the urinary stress incontinence is not only caused by the weakening of the sphincter muscle but is also the result of afferent nerve reflexes." The Examiner also states that "the cryo-induced urethral injury does not represent the complexities found in patients with mixed urge and stress incontinence conditions, which not only involves urethral muscle functions but also nerve stimulation". In this regard, it is respectfully submitted that the Examiner provides no technical basis for his assertion that cryo-induced injury in an animal system does not represent a reasonable animal model for patients having SUI. Thus, it is respectfully requested that the Examiner provide a basis for rejecting this scientifically accepted model.

It is respectfully submitted and supported by the accompanying declaration of Dr. M. Chancellor ("Chancellor declaration") that cryo-induced injury to the urethra in rodents does serve as an art-recognized and accepted system in which to evaluate treatments and therapies for genitourinary tract disorders, such as SUI, in patients having such disorders.

It is also respectfully submitted that applicants' presently claimed invention is supported by the instant specification, which discloses that injection of autologous MDCs, which can differentiate into appropriate muscle cells after injection, as do myoblasts, results in safe and non-immunogenic long-term survival of myofibers in the

lower urinary tract. (Example 4 and Figs. 15A-15C). Applicants' disclosure and the experimental data and results, presented in the accompanying Chancellor declaration and obtained in accordance with applicants' disclosure, reveal that repair of genitourinary tract injury, damage, or dysfunction involving genitourinary muscle tissues, such as bladder, sphincter and urethra can be achieved by introducing MDCs into muscle tissue of the genitourinary tract. The effectiveness of MDCs in such methods is exemplified by accepted animal models in this art. The experiments described in Appendices 1 and 2 of the Chancellor declaration involve MDCs obtained according to the method of applicants' invention and show that continence is improved following urethral injection of MDCs in a rat animal model of urinary incontinence, e.g., stress urinary incontinence (SUI).

In his declaration, Dr. Chancellor describes experiments performed under his direction in which MDCs of the invention are injected into the urethral tissue of rats as a treatment for urinary incontinence related to urethral injury. In the first set of experiments described in Appendix 1 of the Chancellor declaration, cauterization was used to create urethral injury as a model for intrinsic sphincter deficiency (ISD), which is a recognized cause of one type of SUI. The results of the experimental studies described in Appendix 1 demonstrate that MDC injections restore the mean urethral leak point pressure (LPP) of injured animals to control levels by 6 weeks post surgery.

In the second set of experiments described by Dr. Chancellor in Appendix 2 of the declaration, the rat model of urinary incontinence involves urethral denervation. In both the first and second sets of experiments, the presence and bulking of MDCs in the injected tissue over time were monitored by the expression of β-galactosidase in the injected site, as the cells were transduced to contain an exogenous LacZ reporter gene, which was successfully expressed in the MDCs. (See, e.g., the Chancellor application at page 22, lines 19-27; Figs. 15A-15C; page 46, lines 5-28 to page 47, lines 1-28; and page 65).

Also in these studies, the art-recognized parameter of leak point pressure (LPP) was assessed after MDC injection relative to controls as described, to determine a restoration of function to the damaged urethral tissue. LPP indicates the intravesical pressure at which the urethra lacks sufficient tension to prevent urine leakage. Increased LPP equates with increased continence and is an accepted parameter for assessing continence in clinical cases, as well as in animal models of SUI, as discussed by Dr. Chancellor in his declaration. In the pertinent art, LPP is considered to be a defining parameter for continence. (See, for example, the Chancellor declaration at ¶¶8 and 9; the FDA Guide, attached at Tab 3 of the Chancellor declaration; and the attached pertinent pages from a publication stemming from the 2nd International Consultation on Incontinence, namely, P. Abrams et al., (Eds.), 2nd Edition, 2002, *Incontinence*, Plymouth, UK, Health Publication Ltd, attached hereto as Exhibit 1). Applicants submit that a method of injecting MDCs resulting in improved continence as determined by an increase in LPP supplements the support already present in applicants' disclosure for the present claims, which are directed to repairing or ameliorating injury, damage, or dysfunction in genitourinary tract muscle tissue associated with SUI.

In addition, for the experiments described in Appendices 1 and 2 of the Chancellor declaration, histological examination of the urethral tissue from the animals that had received treatment with MDCs revealed the presence of urethral tissue of normal appearance (similar to sham-operated, or untreated animals) versus necrosed tissue in the animals that had received injection of non-MDC cells. Accordingly, the Chancellor declaration, which is fully supported by the teachings of the disclosure of the instant application, provides further evidence that applicants have taught those having skill in the art to make and use the claimed invention without undue experimentation.

As further support for the experiments and results described in the Chancellor declaration, the following papers describe work, based on the teachings of the disclosure of the Chancellor application and performed under Dr. Chancellor's direction,

which shows that urethral injection of MDCs is effective in improving continence in animal models of incontinence:

1. J.Y. Lee et al., 2003, "The effects of periurethral muscle-derived stem cell injection on leak point pressure in a rat model of stress urinary incontinence", *Int. Urogynecol. J.*, 14:31-37, (attached as Exhibit 2).

In the Lee et al. paper, urethral degeneration induced by sciatic nerve denervation is used to model SUI. In experiments employing this model, MDC injections administered in accordance with the present invention were shown to improve continence at 1 and 4 weeks post injury.

2. T. Cannon et al., 2003, "Improved sphincter contractility after allogeneic muscle-derived progenitor cell injection into the denervated rat urethra", *Urology*, 62(5):958-963 (attached as Exhibit 3).

In this paper, Cannon et al. demonstrate that MDC injections aid in the functional recovery of injured urethral sphincter, as evidenced by increases in the muscle contraction amplitudes of isolated urethral strips following MDC treatment. MDC injection resulted in the restoration of function to damaged genitourinary tissue, in addition to acting as passive bulking agents. The results of Cannon et al. provide support for the presently claimed invention, which is directed to repair or amelioration of dysfunctional genitourinary muscle tissue.

The foregoing articles and experimental data, which are based on the invention described in the instant application, effectively demonstrate an improvement of continence following injections of MDCs into urethral tissue. Contrary to the Examiner's position that "[a]t best, the specification only teaches the treatment of urethral injury ... [in] cryo-injured urethra of female rat," applicants maintain that such a model is valid and useful in assessing MDCs of the present invention in the treatment of urinary incontinence. Moreover, the results of numerous research experiments performed on the basis of the instant invention demonstrate the efficacy of MDC injections as

treatments to improve continence in both nerve-degeneration and cautery-injury models of SUI.

The Examiner has also remarked that "... the applicant fails to disclose that bulking of the sphincter muscle alone would lead to the treatment of urinary stress incontinence, since the urinary stress incontinence is not only caused by the weakening of sphincter muscle but is also the result of afferent nerve reflexes." (page 5 of the office action). Applicants respectfully disagree on this point. Those having skill in the art understand that urethral afferent nerve activity does not *cause* stress urinary incontinence. Rather, stress incontinence can induce or increase urethral afferent nerve activity. As it happens, the activation of the urethral afferent nerve reflexes, caused by a weak sphincter muscle, can result in overactive bladder and urge incontinence, as is mentioned in S.Y. Jung et al., 1999, *J. Virol.*, 162:204-212, a copy of which has been previously provided to the Examiner.

Applicants submit that the claimed invention provides a treatment for patients afflicted with genitourinary tract injury or dysfunction associated with urinary incontinence, as well as for patients having mixed incontinence, in which the stress urinary incontinence component of the affliction can be particularly treated. It is not a condition of either the usefulness or the operativeness of the present invention that afferent nerve reflexes be treated as well as sphincter muscle bulking or augmentation.

Applicants also respectfully submit that the Examiner may be misinterpreting the cause-effect relationship between SUI and urge incontinence. While urge incontinence is facilitated by stress urinary incontinence, urge incontinence is not a cause of stress urinary incontinence. In addition, SUI can be treated without modulating the afferent nerve reflexes that are associated with urge incontinence. Stress and urge incontinence can be considered disparate dysfunctions. As stated in the accompanying declaration of Dr. Chancellor, the bulking of the sphincter muscle tissue would lead to a treatment for SUI if sphincter muscle weakening is prevented or ameliorated, in the absence of a concomitant treatment of afferent nerve reflexes.

According to Dr. Chancellor, it is widely recognized and accepted among those having skill in the art that bulking or augmentation of the sphincter is an appropriate and conventional means of treating SUI. Indeed a number of bulking agents, e.g., collagen, microplastique, fat, blood, silicone, microspheres, self-detachable balloon systems and microcarbon particles, have been proposed and used to treat SUI. (*See, e.g.,* FDA Guide, Tab 3 of the Chancellor declaration). It is to be understood that bulking agents work by increasing the outflow resistance of urine from the bladder into the urethra (as quantified by LPP) and not by acting upon afferent nerve reflexes directly.

As maintained by Dr. Chancellor in his declaration, LPP is accepted among clinicians as the objective outcome parameter for determining efficacy of SUI treatments and assessing improvement in continence. For example, the FDA has relied on LPP measurements in guidelines for determining whether collagen and other agents could serve as periurethral bulking agents. A copy of the relevant FDA Guide (November 29, 1995) is provided at Tab 3 of the Chancellor declaration. On this basis, it is assumed that the efficacy of MDCs as a bulking agent would be evaluated on the same criteria. (See the Chancellor declaration, ¶¶8 and 9). Accordingly, the Chancellor declaration and the above articles, which follow the teachings of the instant specification and demonstrate MDC-induced improvements in LPP, serve as acceptable support for the use of MDC injections as treatment in the repair or amelioration of injury, damage, or dysfunction of genitourinary tissue, associated with SUI.

The presently claimed invention is directed to the repair or amelioration of injury, damage, or dysfunction to genitourinary muscle tissue, particularly, urethra, sphincter, or bladder muscle tissue, associated with SUI. The claimed methods involve the introduction of applicants' described MDCs into a genitourinary muscle tissue site, wherein the MDCs exhibit long-term survivability and myofiber formation following injection, as shown by the examples in the instant specification. The MDCs repopulate, bulk up and augment muscle tissue into which they are introduced. See, for example, Example 2, which describes the injection into the urethral wall to treat urethral injury in

a mouse model system. Example 2 demonstrates that injected MDCs survive, as evidenced by being monitored for 30 to 60 days following introduction into the animal needing treatment, and allow the formation of regenerative myofibers in the urethral wall, which is comprised of muscle tissue. Animals that had been treated by MDC injection exhibited increased urethral pressure. Improved urethral contractility is also described in those animals which had injury to the urethra. Thus, the demonstrable formation of new muscle tissue following injection of MDC into injured tissue of the genitourinary tract, such as the urethral wall, supports the claimed invention as a treatment for repair and treatment of genitourinary tract injury, damage, or dysfunction. Example 3 also demonstrates improved contractility of cryo-injured urethral muscle following MDC injection and shows similar improvements in detrusor muscle contractility following MDC injection into cryo-injured bladder muscle.

The instant specification also teaches that the injection of primary rat MDCs according to the applicants' method resulted in a large bulking effect in the urethra wall, as shown in Fig. 15C, which depicts the cross section of a rat urethra after treatment with MDCs. Also, as shown in Figs. 15A, 15B and Example 4, muscle-derived cells according to the invention survived for at least 6 months following injection into mouse bladder and urethra without damage to the bladder wall, and expression of ß-galactosidase encoded by the LacZ gene transduced into the cells prior to injection was maintained at approximately 66% after 70 days. These results support the ability of the MDCs of the invention to persist and to repopulate at the site of injury or damage to genitourinary muscle tissue.

Further, it is respectfully submitted that the application also supports the introduction of a heterologous protein-encoding polynucleotide into MDCs and the expression of the introduced protein by the cells. As specific examples, using the ß-galactosidase reporter gene expression system, applicants demonstrated that IRAP and iNOS products were expressed by muscle-derived cells into which a vector containing a polynucleotide encoding these respective products was introduced. (See, e.g., pages 20 and 21, Figs. 8A-8D; page 31, lines 17-28 to page 32, lines 1-17, and

Example 6, pages 72-73 and Example 7, pages 72-76). Thus, the muscle derived cells of the presently claimed invention are able to express heterologous proteins from polynucleotides encoding such proteins introduced in a vector, and the expression is maintained over time following injection of these cells into a muscle tissue site as determined by monitoring of ß-galactosidase expression in the injected cells.

In sum, it is thus respectfully submitted that the presently claimed invention sufficiently and effectively supports the repair of injured, damaged, or dysfunctional sphincter, bladder, or urethral muscle of the genitourinary tract following introduction of MDC into the relevant tissue. As presented in the accompanying Chancellor declaration, experiments conducted after the filing of the instant application, and carried out based on the teachings of this application, show the beneficial effects of MDC for treating incontinence.

Thus, applicants' submit that the claims as presented herein allow the skilled artisan at the time of the instant invention to make and use the invention as presently claimed without undue experimentation. Accordingly, withdrawal of the §112, first paragraph rejection is respectfully requested.

II. The Indefiniteness Rejection

Claims 119-195 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The Examiner has indicated that the term "muscle derived cells" in the relevant claims is not defined by the claim.

The newly presented claims clearly describe a process to obtain the MDC population that produces diverse types of muscle cells for use in the claimed methods as taught in the disclosure of the instant specification. (See, e.g., page 42. lines 10-28 to page 43, lines 1-2; page 67, lines15-28 to page 68. lines 1-6; and page 98, lines 21-28 to page 99, lines 1-10). Applicants respectfully submit that the present claims describe and define with clarity the muscle derived cells (MDCs) of the invention such that one of ordinary skill in the art is able to reasonably ascertain the scope of

applicants' claimed invention. Accordingly, reconsideration and withdrawal of the §112, second paragraph rejection are respectfully requested.

CONCLUSION

Applicants respectfully submit that the presently pending claims are in condition for allowance, and an action progressing this application to issue is courteously urged.

Should any fees additional to those paid herewith be deemed to be properly assessable during the pendancy of this application, or for the timely consideration of this Amendment, the Commissioner is hereby authorized to charge any such additional fee(s), or to credit any overpayment, to Deposit Account No. 08-0219, Order No. PIT-010.

In the event that the Examiner is of the opinion that further discussion is necessary, the Examiner is respectfully requested to telephone the applicant's undersigned representative at (212) 937-7315.

Respectfully submitted,

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